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Unleashing Cardiopoiesis: A Novel Role for G-CSF

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Identifying pathways for cardiac muscle creation is a paramount objective of cardiac stem cell biology. In this issue of *Cell Stem Cell*, Shimoji and colleagues (2010) report the unforeseen ability of granulocyte colony-stimulating factor (G-CSF) to drive cardiopoiesis in mouse, primate, and human pluripotent cells.

Cardiovascular disease accounts for more than a third of mortality in high-income countries, the commonest form being ischemic heart damage (myocardial infarction) resulting from obstructed coronary arteries. Cardiomyocyte death without equivalent regeneration also is typical of cardiomyopathies. Rescuing cardiac muscle cell number is thus opportune as one means to rescue the heart's performance as a biomechanical pump. A priori approaches include preventing apoptosis, stimulating survival pathways, activating endogenous progenitors within the heart (which, unassisted, are insufficient to restore the organ), and cell grafting. More than 20 clinical trials of cell therapy for heart repair have been reported worldwide, chiefly with bone marrow cells, whose benefits are better explained by blood vessel formation and secreted factors than by the transdifferentiation once envisioned (Segers and Lee, 2008). In contrast, the capacity to generate new cardiac muscle is unquestionable for embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and progenitor/stem cells from the heart itself. Nevertheless, problems remain: if cardiopoiesis is to be more than a rare unguided event, there exists a compelling need to pinpoint the signals and networks that drive the necessary cardiac lineage decisions or expansion of committed or differentiated cells. This task is made more complex by the varying and even dichotomous roles factors play at different developmental stages in vivo and in differentiating ESCs, such as Wnts and bone morphogenetic proteins that can alternatively induce or repress cardiopoieisis (Olson and Schneider, 2003; Yuasa et al., 2005).

Building upon the previously reported ability of Noggin, a bone morphogenetic protein inhibitor, to stimulate cardiac myogenesis in differentiating ESCs (Yuasa et al., 2005), Shimoji and colleagues identified the G-CSF receptor, *gcsfr3*, as a Noggin-induced gene that is enriched in developing mouse myocardium and tested its utility as a potential target to stimulate cardiopoiesis (Shimoji et al., 2010). Expression of the G-CSF receptor and its ligand was enriched in embryonic cardiac myocytes at E9.5, peaking at E10.5 and E12.5, respectively. Intrauterine administration of G-CSF on E9 increased cardiomyocyte proliferation. Strikingly, a single dose of G-CSF at E9 conferred a persistently enlarged trabecular layer and increased thickness of the compact layer in late gestation and neonatal hearts. This effect was abrogated in *gcsfr3*$^{-/-}$ mice, which displayed decreased proliferation of cardiomyocytes during development, a thin-walled hypoplastic heart, and 50% intrauterine mortality. The effect on cycling in culture was marked with cardiomyocytes isolated at E9.5, minimal in cardiomyocytes from E12.5 embryos, and unseen in those from neonates.

Next, it was postulated that the G-CSF receptor could be exploited in ESC-derived cardiomyocytes. Indeed, G-CSF increased the incidence of beating mouse embryoid bodies 5-fold. The normal upregulation of cardiac markers was abrogated by neutralizing antibodies to G-CSF. G-CSF did not alter the downregulation of Brachyury/T, a key marker of primitive mesoderm, or the initial appearance of Nkx2.5, the cardiogenic homeodomain transcription factor. Rather, G-CSF specifically increased later levels of Nkx2.5, suggesting a mitogenic effect on committed progenitors rather than an inductive one, an inference substantiated by the incorporation of BrdU by cells expressing the cardiogenic transcription factor Mef2c. Remarkably, as the net result, most cells in G-CSF-treated cultures expressed the cardiac proteins examined.

Lastly, the authors tested the impact of G-CSF on cardiomyogenesis in marmoset ESCs and human iPSCs. In both cases, G-CSF increased the percentage of beating embryoid bodies, upregulating cardiac transcription factors and markers of later cardiac differentiation (e.g., NKX2.5, TBX5, MYL2, MYH6, and NPPA in hiPSCs).

In summary, the authors report a novel mitogenic role for G-CSF in cardiac lineage-committed cells, with potential importance for generating nascent cardiomyocytes efficiently from ESCs, iPSCs and, conceivably, heart-derived cardiac progenitor cells. Toward these ends—not only for therapeutic grafting but also other applications requiring human heart muscle, like toxicology screens, disease models, and drug discovery—it will be important to learn how G-CSF affects specific cardiomyocyte subpopulations (atrial, ventricular, conduction system) and to determine its impact on biophysical and pharmacological maturation. G-CSF signaling has been investigated chiefly in the hematopoietic system and much remains to be learned in its role as a cardiopoietin. Activation of G-CSFR triggers multiple pathways involving JAK/STAT, Ras/Mek/Erk, Src-related kinases, Akt, and SOCS3 (Panopoulos and Watowich, 2008). In the current study, G-CSF and JAK2/STAT3 drove proliferation of cardiomyocytes in embryoid bodies and embryonic hearts, whereas G-CSF and JAK/STAT promoted cardiomyocyte survival, not proliferation, in adult mice after infarction (Harada et al., 2005), differences presumably reflecting age or pathobiological context.

Human trials of G-CSF for heart repair have already been undertaken, facilitated

by its prior use in autologous bone marrow stem cell transplantation. Two broadly different perspectives are recognizable, focused on cardiac myocytes, as above, or more typically on bone marrow-derived cells' mobilization and homing to injured hearts. Alone, G-CSF impairs homing of bone marrow cells to the heart, perhaps by inhibiting the migratory response to SDF-1; by contrast, G-CSF plus a CD26/dipeptidylpeptidase IV inhibitor that elevates SDF1 levels improved these cells' recruitment to the heart, augmenting vascularization, pump function, and survival (Zaruba et al., 2009). Cardiac-resident progenitor cells are a third potential target, as indicated by the fact that G-CSF may enhance the number of cardiac Sca-1$^+$ cells (Brunner et al., 2008; cf. Harada et al., 2005). Is G-CSFR functionally coupled in the endogenous cardiac progenitor/stem cells resident in adult human hearts? Are salutary effects of G-CSF on the adult heart mediated in part by these cells? Might G-CSF be used to drive cardiopoiesis by adult cardiac progenitor cells, in culture or in situ?

Optimization of any potential cardiopoietin must take into account the effects on proliferation as well as lineage commitment. Proliferation can occur in embryonic cardiomyocytes (Olson and Schneider, 2003), and Shimoji et al. (2010) localized the proliferation evoked by G-CSF to Mef2$^+$ cells, Nkx2.5$^+$ cells, and α-actinin$^+$ cells. However, susceptibility to G-CSF was largely lost by E12.5. It remains to be learned whether other hypoplastic cardiac phenotypes impinge on this axis, e.g., through precocious differentiation, whether cardiac progenitors from both heart fields require G-CSF, and whether the effect of G-CSF is entirely myocyte autonomous.

The unexpected cardiac-lethal phenotype of *gcsfr3*$^{-/-}$ mice differs from the original report of this knockout line, which had no premature lethality and was said to develop normally (Liu et al., 1996). This inconsistency might be explained by the differing genetic background (ultimately backbred to C57Bl/6), but leads to the more general consideration, how many other essential cardiopoietins have been overlooked in ostensibly conclusive models? How might heart-forming factors best be identified in the future? Complementary, higher-throughput approaches to detect novel cardiopoietins have begun to include robotic screens in stem cells and other systems (Sadek et al., 2008). Along with other "high-bandwidth" approaches like saturation mutagenesis, these experimental platforms hold the promise of defining workable triggers for cardiac muscle creation, beyond the insights obtainable in stem cells or model organisms manipulating just one factor or pathway at a time, on an artisanal scale.

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Crossing Boundaries: Direct Programming of Fibroblasts into Neurons

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In a recent paper in *Nature*, Vierbuchen et al. (2010) show that fibroblasts can be directly converted into functional neurons by defined factors. This finding sheds new light on the biology underlying cell-fate restrictions and might offer a new avenue for studying neurological diseases.

The finding that differentiated somatic cells such as fibroblasts can be reprogrammed into induced pluripotent stem cells (iPSCs) by four (or even fewer) transcription factors (TFs) revolutionized the understanding of cellular plasticity and provided a novel tool to study developmental processes and mechanisms of human disease (Takahashi et al.,